

## The Association of CAG Trinucleotide Repeats of *Androgen Receptor* Gene with the Incidence of Castrate Resistant Prostate Cancer in Javanese Population

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### ABSTRACT

Androgens play an important role for prostate cells proliferation, survival and development of prostate cancer (PCa). Androgen ablation therapy is a common procedure to treat PCa. However, in most of the cases PCa relapse after 18-24 months of therapy and develop into castration resistant prostate cancer (CRPC). Polymorphism in *Androgen Receptor* (AR) gene has been known as a risk factor for PCa and CRPC. This study aims to evaluate the association of the length of CAG repeats within AR with the incidence of CRPC in Javanese population, in Indonesia. Forty two patients enrolled in this study, genomic DNA was extracted from peripheral blood leukocytes, PCR was performed to amplify exon 1 of AR and genotyping of CAG repeats was performed by Sanger Sequencing. Of the 42 patients, 17 were excluded from the study due to incomplete data after 9 months of observation. Of the 25 remaining patients, three of them (12%) experienced tumor-relapse and developed CRPC phenotype after hormonal therapy. The mean of PSA levels were 277.5 and 240.7 mg/dL for CRPC patients and non-CRP patients respectively ( $p=0.886$ ). We observed that the CRPC patients tend to have shorter CAG repeats (22) than those of non-CRP patients (24) ( $p=0.878$ ). Whereas the mean of Gleason score of CRPC patients (7) were slightly higher than those of non-CRP patients (6,3) ( $p=0.859$ ). Although not significant, Kaplan Meier curve analysis showed that the CAG repeats lower than 22 repeats had a better survival compared to those who have CAG repeats longer than 22. In conclusion, there was no significant association between the lengths of CAG repeats with the incidence of CRPC in Javanese population, however we observed that CRPC patients tend to have shorter length of CAG repeats, lower Gleason score and higher PSA levels pre-ADT (androgen deprivation therapy) compared to those of non-CRP patients.

**Keywords:** *Androgen Receptor* (AR), CAG repeats, CRPC, Gleason score, PSA

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### INTRODUCTION

The prevalence of prostate cancer (PCa) in Caucasian is quite high, around 25,000 and 230,000 new cases were identified per-year in UK and USA respectively. Despite the increase of awareness for screening and early diagnosis of PCa, still this cancer is the second highest cause of death in men in Western countries<sup>1</sup>. Prostate cancer relies on Androgens signaling pathway to proliferate, survive and metastasized<sup>2,3</sup>. Hence, in most of the cases PCa can be successfully treated with Androgen deprivation therapy (ADT) which can be achieved through surgery (Orchiectomy) or drugs that suppress androgen production by using luteinizing hormone-releasing hormone (LHRH) agonists. This treatment can

also be combined with the administration of Androgen Receptor (AR) antagonist to obtain the maximum androgen blockade. Although in most of the cases (80%) the initial response to androgen deprivation therapy is good, but nevertheless most of the patients relapsed after 12-24 months which is characterized by increased levels of circulating Prostate-Specific Antigen (PSA) as an indicator of regrowth of PCa that have adapted to hormone-deprived environment<sup>4-5</sup>. This new growth tumor that survives independent of Androgens is also known as Castration Resistant Prostate Cancer (CRPC). Themetastasize CRPC has poor survival rate, the average is around 16-18 months<sup>6</sup>. This high mortality rate is



Table 3: The Average of CAG length in CRPC and nCRPC patients

	CRPC n = 3	n CRPC n = 22	P-value*
CAG repeat (Average)	22	24	0.24

CRPC: Castrate Resistant Prostate Cancer, nCRP: non-Castrate Resistant Prostate Cancer, \*: T-test

Table 4: The Average of Gleason Score in CRPC and nCRPC patients

	CRPC N = 3	nCRPC N = 22
Gleason Score (Average)	7	6.32

CRPC: Castrate Resistant Prostate Cancer, nCRP: non-Castrate Resistant Prostate Cancer, \*: T-test

from all patients and the study was approved by the ethics committee of the Faculty of Medicine, University of March, Central Java, Indonesia. We conducted an observational study with these 42 prostate cancer patients for nine months.

#### DNA Isolation

DNA was extracted from peripheral blood leukocytes of patients using genomic DNA isolation kit genome (Roche Life Sciences) in Magna Pure 32 LightCycler instrument (Roche Life Sciences) according to the manufacturer's protocol.

#### Genotyping CAG Repeat

Polymerase Chain Reaction (PCR) was performed using 50 µl containing 100 ng of DNA, 25 µl ready to use PCR mix KAPA2G PCR Fast kit (Kapa biosystem, Wilmington, Massachusetts, USA) and 1 µl of 10 pmol of each primer forward 5'ACTACCGCATCATCACAGCC 3' and primer reverse 5'CTTAAGCCGGGAAAGTGG 3'. PCR was performed with touch down PCR program for 35 cycles with the annealing temperature from 68-58°C. The DNA denaturation, primer annealing and the extension of DNA were conducted for one minute in each cycle. The last step of PCR was the extension step at 72°C for 10 minutes. Five microliters of the PCR product was checked on 2% of Agarose gel. The remaining of the PCR product was used for Sanger Sequencing the Big Dye Terminator v3.1 Sequencing Kit (Applied Biosystems, Foster City, USA) in an automatic sequencer ABI 3730XL. The length of CAG repeats is determined by counting manually the number of CAG repeats in the electropherogram of Sanger Sequencing using Bioedit software.

#### Measurement of Prostate Specific Antigen (PSA)

The level of PSA was measured by using ARCHITECT Total PSA assay (ARCHITECT reagent kit, Abbot, Ireland) according to the manufacturer's protocol.

#### Statistical analysis

To evaluate the rate of survival of subjects with different CAG repeat visually used Kaplan-Meier curves. To test the survival rate of the comparison between the two

populations with different number of CAG repeats statistical test Log-Rank was used using RStudio software version 0.99.442 and survival package. The difference in the proportion of CRPC and non-CRPC was analyzed using fisher exact in EpiInfo software version 7.1.5.2. The p-value < 0.05 was considered as significant.

## RESULTS

Of the 42 patients who enrolled in this study, eight patients passed away and nine patients were excluded due to incomplete data after 9 months of observation. Of the 25 remaining patients, three of them were observed to have CRPC phenotype (12%). In all CRPC patients the recurrent occurred in 3 months after Androgen deprivation therapy. The mean of PSA level of CRPC was higher (277.5 mg/dL) than those in non-CRPC patients (240.7 mg/dL) (Table 1). The Sanger Sequencing electropherogram of CAG repeat in exon 1 of AR was shown in Figure 1. The length and the mean of CAG repeats number of CRPC patients (22 repeats) was lower than those of non-CRPC patients (24 repeats) (Table 2 and 3), whereas the mean Gleason score of CRPC patients (7) was higher than those of non-CRPC patients (3) (Table 4). Survival analysis showed that patients with shorter CAG repeat (< 22) had better survival compared to those with long CAG repeat (>22) (Figure 2).

## DISCUSSION

Androgen deprivation therapy by using Androgen suppression or anti-Androgen is a common procedure to treat PCa. This therapy leads to the decline of the tumor growth, hence improving the quality of life of PCa patients. However, the effect of this therapy in most cases is transient. After 18-24 months of Androgen deprivation therapy, the tumors are re-grown and develop into castration resistant prostate cancer (CRPC) also known as Androgen-independent PCa. In this current study the incidence of CRPC is around 12% (3/25 patients). Two of these patients have CAG repeats lower than 22 repeats, while one has 25 of CAG repeats, the average of CAG repeats in CRPC patients is 22 while in non-CRPC is 24 (Table 3). This result is similar to the previous study performed by Mittal, et al, which showed that CRPC patients tend to have short CAG repeats (20) than that in non-CRPC patients (22)<sup>21</sup>. As it was expected, in this study the average of PSA level of CRPC patient was higher than that of non-CRPC patient (277.5 Vs 240.7 mg/dL) as well as Gleason score (7 vs 6.3). There was no significant different of survival rate between CRPC and non-CRPC patients in this population.

CAG repeats is located in the N-terminal of AR which is known to be critical for interaction with other co-regulator proteins to activate the expression of AR target genes. The short length of CAG repeats (<22) has been known to be able to enhance the transactivation activity hence inducing the Androgen signaling pathway. This process is believed to contribute to the development of PCa and it has been proven that the short length of CAG repeats (<22) is associated with the incidence of PCa in

some population. However, activation of Androgen signaling pathway occurs in many different mechanism. In CRPC for example, although Androgens-ablation therapy could successfully decrease the total serum Testosterone level hence decreasing the Androgen signaling pathway and eventually could stop tumor growth, however Androgen-deprived environment seems to not disturb the Androgen signaling pathway in CRPC as AR and AR target genes have been shown to be up-regulated. One of the mechanisms is via AR gene amplification<sup>21, 22</sup>. Previous studies also showed that AR could activate by alternative steroidal molecules including estrogens, corticosteroids and progesterone<sup>23,24</sup>. As the length of CAG repeats was not associated with the incidence of CRPC in Javanese population, it seems that the up-regulation of Androgen signaling mainly not occur via shortened of CAG repeats. Another reason could be that this study was conducted with limited number of patients (n=25), hence we lack the power to statistically calculate the association of the length of CAG repeats with the incidence of CRPC in Javanese population. Study with bigger number of samples need to be performed to have more solid and accurate results.

#### CONCLUSION

There was no significant association between the length of CAG repeats with the incidence of CRPC in Javanese population. However, it was observed that CRPC patients tend to have higher PSA levels, higher Gleason score and shorter CAG repeats length compare to those non-CRPC patients.

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#### REFERENCES

- Schulz WA, Burchardt M, Cronauer MV Molecular biology of prostate cancer. *Mol Hum Reprod* 2003; 9: 437-448.
- Heinlein CA, Chang C. Androgen Receptor in Prostate Cancer. *Endocrine Rev* 2002; 25(2): 276-308.
- Edwards J, Bartlett JM. The androgen receptor and signal-transduction pathways in hormone-refractory prostate cancer part 2: androgen receptor cofactors and bypass pathways. *BJU Int* 2005; 95: 1327-35.
- Heinlein CA, Chang C. Androgen receptor in prostate cancer. *Endocrine Reviews* 2004; 25(2): 276-308.
- Trachtenberg J, Blackledge G. Looking to the future: Advances in the management of hormone-refractory prostate cancer. *Eur Urol Suppl* 2002; 1: 44-53.
- Marques RB, Dits NF, Erkens-Schulze S, van Weerden WM, Jester G. Bypass mechanism of the androgen receptor pathway in therapy-resistant prostate cancer cell models. *Plos One* 2010; 5: e13500.
- Gelmann EP. Molecular Biology of the Androgen Receptor. *J Clin Oncol* 2002; 20: 3001-3015.
- Koochekpour S. Androgen receptor signaling and mutations in prostate cancer. *Asian J Androl* 2010; 12: 639-657.
- Nelson KA, Witte JS. Androgen receptor CAG repeats and prostate cancer. *Am J Epidemiol.* 2002; 155: 883-890.
- Chamberlain NL, Driver DE, Miesfield RL. The length and location of CAG trinucleotide repeats in the androgen receptor N-terminal domain affect transactivation function. *Nucl Acids Res* 1994; 22: 3181-3186.
- Tut TG, Ghadessy FJ, Trifiro MA, Pinsky L, Yong EL. Long polyglutamine tracts in the androgen receptor are associated with reduced trans-activation, impaired sperm production, and male infertility. *J Clin Endocrinol Metab* 1997; 82: 3777-3782.
- Sartor O, Zheng Q, Eastham JA. Androgen receptor gene CAG repeat length varies in a race-specific fashion in men without prostate cancer. *Urology* 1999; 53: 378-380.
- Bentmar HM, Giwercman A, Bjartell A, et al. Androgen receptor polymorphism dependent variation in prostate specific antigen concentrations of European men. *Cancer Epidemiol Biomarkers Prev* 2014; 23(10): 2048-2056
- Madjunkova S, Eftimova, Georgiev V, Petrovski D, Dimovski A, Plaseska-Karanfilska D. CAG Repeat Number in the Androgen Receptor Gene and Prostate Cancer. *Balkan J Med Genet* 2012; 5: 31-36.
- Zeegers MP, Kiemeny LA, Nieder AM, Ostrer H. How strong is the association between CAG and GGN repeat length polymorphisms in the androgen receptor gene and prostate cancer risk? *Cancer Epidemiol Biomarkers Prev* 2004; 13: 1765-1771.
- Huang SP, Chou YH, Chang WS, et al. Androgen receptor gene polymorphism and prostate cancer in Taiwan. *J Formos Med Assoc* 2003; 102: 680-686.
- Sircar K, Gottlieb B, Alvarado C, et al. Androgen receptor CAG repeat length contraction in diseased and non-diseased prostatic tissues. *Prostate Cancer Prostatic Dis* 2007; 10: 360-368.
- Mononen N, Ikonen T, Autio V, et al. Androgen receptor CAG polymorphism and prostate cancer risk. *Hum Genet* 2002; 111: 166-171.
- Suzuki H, Akakura K, Komiya A, et al. CAG polymorphic repeat lengths in androgen receptor gene among Japanese prostate cancer patients: Potential predictor of prognosis after endocrine therapy. *Prostate* 2002; 51: 219-224.
- Giovannucci E, Stampfer MJ, Krithivas K, Brown M, Brufsky A, Talcott J, Hennekens C H, and Kantoff, PW. The CAG repeat within the androgen receptor gene and its relationship to prostate cancer. *Proc Natl Acad Sci* 1997; 94: 3320-3323.
- Mittal RD, Mishra DK, Mandhani A. Role of an androgen receptor gene polymorphism in development of hormone refractory prostate cancer in Indian

- Population. *Asian Pacific J Cancer Prev* 2007; 8: 275-278.
22. Visakorpi T, Hyytinen E, Kolvisto P, Tanner M, Keinänen R, Pääbo C, et al. In vivo amplification of the androgen receptor gene and progression of human prostate cancer. *Nat Genet* 1995; 9: 401-406.
23. Ruiz C, Lenkiewicz E, Evers L, Holley T, Robeson A, Kiefer J, et al. Advancing a clinically relevant perspective of the clonal nature of cancer. *Proc Natl Acad Sci* 2011; 108: 12054-12059.
24. Zhao X, Malloy PJ, Krishnan AV, Swami S, Navone NM, Peehl DM., et al. Glucocorticoids can promote androgen-independent growth of prostate cancer cells through a mutated androgen receptor. *Nat Med* 2002; 6: 703-706.